Supporting Information

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SI Procedures and Methods

Cultivation of *P. falciparum*. Blood stages of *P. falciparum* strains Dd2 and K1 (CQ-resistant) as well as HB3 and 3D7 (CQ-sensitive) were maintained in culture using a modified protocol of Trager and Jensen (1). RPMI medium 1640 supplemented with $NaHCO_3$ and Hepes (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), pH 7.4, 20 μg/ml gentamicin sulfate, 2 mM glutamine, 200 μM hypoxanthine, 0.2% Albumax II, and 4.1% human serum were used for cultivation. Washed human erythrocytes of blood group A+ were added to a hematocrit of 3.3%. Parasites were maintained at a parasitemia of 1 to 10% in an atmosphere of 90% N_2/5% O_2/5% CO_2 at 37 °C and synchronized to the ring stage using the sorbitol method (2).

Preparation of Protein Extracts. The cell culture and synchronizing procedures were conducted according to a time-controlled procedure. Life cycle determination, saponin-lysis, parasite lysis, and sample preparation were performed as previously described (3, 4). Cultures to be treated were incubated with 3 × IC_{50}-concentrations of CQ at 26 to 28 h postinfection in the trophozoite stage and harvested after 6 h. Briefly, harvested infected RBCs were lysed by saponin treatment (7 mM K_2HPO_4, 1 mM NaH_2PO_4, 11 mM $NaHCO_3$, 58 mM KCl, 56 mM NaCl, 1 mM $MgCl_2$, 14 mM glucose, 0.02% saponin, pH 7.5) for 10 min at 37 °C. After centrifugation, the saponin lysate of infected erythrocytes was used for LC-MS analysis. The parasite pellet was washed 3 times with saponin-containing buffer and once with PBS. The resulting parasite cell pellet was lysed by freezing and thawing and by ultrasonication. The supernatant, after centrifugation at 100,000 × g for 30 min, was used as parasite extract for analysis. For IP experiments, parasites were lysed under argon, where trophozoite pellets in degassed PBS containing 0.2% Triton-X100 were disrupted by 3 cycles of freezing and thawing.

Two-Dimensional Gel Electrophoresis. Two-dimensional electrophoresis was performed according to standardized protocols (5). One-hundred micrograms of each sample to be used for analytical gels was solubilized in buffer containing 6 M urea, 2 M thiourea, 4% wt/vol CHAPS, 75 mM DTT, 0.5% vol/vol ampholytes (GE Healthcare) and Orange G in traces. For preparative gels to be analyzed by MS, 750 μg of protein extract was applied per immobilized pH gradient (IPG) strip. After rehydration in IPG strips, pH 4–9 for 18 h, the IEF was carried out with an IPGphor (GE Healthcare), with a low initial voltage and then by applying a voltage gradient up to 3,500 V, with a limiting current of 50 mA/strip at 20 °C. The total product time voltage applied was 80,000 Vh/strip. For the second dimension, the IPG strips were equilibrated for 30 min in a solution of 6 M urea, 2% wt/vol SDS, 1% DTT, 30% vol/vol glycerol, 375 mM Tris-HCl (pH 8.8), traces of bromophenol blue. The IPG strips were then transferred to 12% SDS/PAGE gels. After the second dimension, the analytical gels to be used for comparative image analyses were stained with silver, according to Heukeshoven (6). Preparative gels were stained with colloidal Coomassie solution containing 0.08% wt/vol G250, 1% vol/vol phosphoric acid, 8% wt/vol ammonium sulfate, 20% vol/vol methanol. To generate statistically valid data, 5 replicates for each condition were produced for analytical gels.

In Silico Gel Image Analysis. The 2DE gels were scanned with an ImageScanner (GE Healthcare) and subjected to image analysis with the ImageMaster 2D Elite v3.10 software (GE Healthcare). The spot maps of analytical gels and preparative gels of all samples were edited. Analytical gels of each sample's group were matched to a reference gel of its group and spot volumina (spot area × intensity) of all gels of a group were normalized to the total spot volume to remove nonexpression-related differences. Regulation of hPrx-2 was then determined by comparing the theoretically derived master gels of each sample group after they have been matched to one another. Determination of spot regulation was achieved by calculating ratios of average, normalized spot volumina [(spot area × spot intensity treated)/(spot area × spot intensity control)].

Organellar Fractionation of FVs. Preparation of FVs and residual parasite lysate was achieved according to Jackson et al. (7). Briefly, trophozoite-infected erythrocytes were cultivated, harvested, and lysed with saponin. The saponin supernatant was centrifuged at 100,000 × g, 4 °C, 30 min to obtain the erythrocytic membranes, resuspendend in an equivolume of PBS, 4 °C, and then used for immunoblots and acetylcholine esterase assays. FVs were prepared from parasites by suspending in ice-cold water, pH 4.5, and triturating through a 27-G 1.2-cm needle. After centrifugation, the supernatant was used as a cytosolic fraction in the immunoblots. The pellet was further disrupted by trituration in 2 mM $MgSO_4$, 100 mM KCl, 10 mM sodium phosphate, pH 7.4, containing 10 ml of 5 mg ml^{-1} DNase 1 (Roche) and collected by centrifugation through 42% Percoll containing 0.25 M sucrose, 1.5 mM $MgSO_4$, pH 7. The final brown pellet, containing the purified vacuoles, was washed once with PBS and then resuspended in 100 μl of PBS. This suspension was used for acetylcholine esterase assays to monitor the purification of food vacuoles (Table S1) (8). For immunoblotting analyses, vacuoles were lysed by 3 cycles of freezing and thawing, followed by 5 strokes of ultrasonication on ice. For immunoblots, 30 μg of vacuolar proteins and cytosol were applied.

Cloning and Expression. Recombinant His-tagged redox proteins from *P. falciparum* (*Pf*Trx1, *Pf*TrxR, *Pf*TPx1) and man ($hTrx^{C73S}$) were produced and purified as described previously (9–11).

The human thioredoxin dependent 2-Cys-peroxidase (hTPx1, hPrx-2, Uniprot Accession Number P32119) was amplified from a human cDNA library using OhTPx1N (5′ GCGC[UNDERLN]GGATCC[/UNDERLN]GCCTCCGGTAACGCGCGCATC 3′) and OhTPx1C (5′ CGCG[UNDERLN]AAGCTT[/UNDERLN]CTAATTGTGTTTGGAGAAATATTCC 3′) as primers for PCR (restriction sites added for cloning are underlined). The PCR-program used was 94 °C for 2 min, followed by 30 cycles of 94 °C, 30 sec, 61 °C, 45 sec, and 72 °C for 1 min, finalized by 4 min at 72 °C. The PCR product obtained (614 bp in size) was ligated into the expression vector pQE30 (Qiagen) with Quick Ligase (New England Biolabs) using the introduced BamHI and HindIII sites, and confirmed by sequencing. For expression of pQE30/hTPx1, *Escherichia coli* M15 (Qiagen) cells were used. Recombinant hPrx-2 was expressed in *E. coli* strain M15 (Qiagen) and purified via Ni-NTA affinity chromatography. Samples were analyzed by reducing SDS/PAGE, and protein concentrations of the fractions were determined measuring the absorbance at 280 nm ($\varepsilon_{280\ nm}$ = 21.6 $mM^{-1}cm^{-1}$).

Enzymatic Thioredoxin Peroxidase Assays. The activities of hPrx-2 and *Pf*TPx1 were measured using thioredoxins from both or-

ganisms as reducing substrates for both Prxs. Thioredoxin peroxidase assays were performed at 25 °C, as described previously with slight modifications (9). An assay containing 50 mM Hepes, pH 7.2, 100 μM NADPH, 200 mU/ml recombinant *Pf*TrxR (determined with *Pf*Trx1 as substrate), and 20 μM recombinant *Pf*Trx1 or hTrx^{C73S} was preincubated to fully reduce the thioredoxin substrate. A baseline was recorded after the addition of 0.1-mM hydroperoxide substrate (hydrogen peroxide, *tert*-butylhydroperoxide, or cumene hydroperoxide). The assay was started by adding 0.13 μM *Pf*TPx1 or 0.38 μM hPrx-2 and the consumption of NADPH was monitored spectrophotometrically ($\varepsilon_{340\ nm}$ = 6.22 $mM^{-1}cm^{-1}$).

Assays in parasite extract were performed with degassed solutions under argon to prevent overoxidation of hPrx-2 (this step is not necessary when working only with recombinant proteins, as above). Assays were performed as described above under following conditions: preincubation of buffer (50 mM Hepes, 1 mM EDTA, pH 7,2), NADPH (100 μM), *Pf*TrxR (200 mU/ml), *Pf*Trx1 (50 μM), and parasite extract 1 μg/μl at 25 °C. A baseline was recorded after preincubation. As peroxide substrate tBOOH (200 μM) was used to start the reaction, the consumption of NADPH was monitored spectrophotometrically.

Indirect IFA. Confocal laser scanning immunofluorescence was carried out using *P. falciparum* CQ-resistant (Dd2) and CQ-sensitive (HB3) parasites fixed with 5% methanol/95% acetone. Primary antibodies were diluted as follows: mouse anti-*Pf*SBP1 (1:300) (13), and mouse anti-GFP (1:200). Three different polyclonal antibodies were used for the detection of hPrx-2 (hTPx1) to exclude cross-reactions and artifacts: rabbit anti-hTPx1, recognizing the peptide L^{103}LADVTRRLSED^{114} of the protein (Axxora) (1:500), rabbit anti-hTPx1 (kindly provided by L. Poole, Wake Forest University School of Medicine, Winston-Salem, NC) (1:100), rabbit anti-peroxiredoxin-SO_3, recognizing active site sulfinic and sulfonic forms (Acris Antibodies, 1:100). Corresponding secondary antibodies conjugated with either Alexa 488 or 546 (Invitrogen) were used at a dilution of 1:1,000. Nuclei of parasites were visualized by staining with Hoechst 33342. For the detection of GFP, a transfected clone expressing cytosolic GFP was used (12). Slides were mounted with mowiol (Calbiochem) and viewed using an LSM510 laser scanning confocal microscope with a Zeiss Axiovert 100 microscope (Carl Zeiss). Images were obtained in succession (multitrack mode). The used Zeiss objective lense has a magnification of 63×, oil-corrected with a numeric aperture of 1.4. Images were acquired with the Zeiss LSM510 v3.2 software.

Multidimensional Protein Identification technology. Precipitated protein preparations of parasite extract and extract from infected RBCs were dissolved in digestion buffer, digested with trypsin and LysC, and analyzed by LC/LC/MS/MS according to published protocols (13, 14). Approximately 100 μg of protein was used for a 6-step for soluble samples and 12-step for insoluble LC/LC/MS/MS analysis on a LTQ-Orbitrap (ThermoElectron). All samples were analyzed in duplicate. The obtained MS/MS spectra were analyzed with SEQUEST 2.7 (15, 16) using a nonredundant *Plasmodium* database (PlasmoDB version 5.0) (17) and a human database (NCBI, August 2008). The SEQUEST outputs were analyzed by DTASelect 2.0 (18). DTASelect 2.0 uses a quadratic discriminant analysis to dynamically set XCorr and DeltaCN thresholds for the entire data set to achieve a user-specified false-positive rate (5% in this analysis). The false-positive rates were estimated by the program from the number and quality of spectral matches to the decoy database (19). Semiquantitative analysis was performed by calculating ratios of spectrum counts of the desired peptides.

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Spot 1:

1	11	21	31	41	51	61	71
MASGNARIGK	PAPDFKATAV	VDGAFKEVKL	SDYKGKYVVL	FFYPLDFTFV	CPTEIIAFSN	RAEDFRKLGC	EVLGVSVDSQ
81	91	101	111	121	131	141	151
FTHLAWINTP	RKEGGLGPLN	IPLLADVTRR	LSEDYGVLKT	DEGIAYRGLF	IIDGKGVLRQ	ITVNDLPVGR	SVDEALRLVQ
161	171	181	191				
AFQYTDEHGE	VCPAGWKPGS	DTIKPNVDDS	KEYFSKHN				

The matched peptides cover **45%** (91/198 AA's) of the protein.

Spot 2:

1	11	21	31	41	51	61	71
MASGNARIGK	PAPDFKATAV	VDGAFKEVKL	SDYKGKYVVL	FFYPLDFTFV	CPTEIIAFSN	RAEDFRKLGC	EVLGVSVDSQ
81	91	101	111	121	131	141	151
FTHLAWINTP	RKEGGLGPLN	IPLLADVTRR	LSEDYGVLKT	DEGIAYRGLF	IIDGKGVLRQ	ITVNDLPVGR	SVDEALRLVQ
161	171	181	191				
AFQYTDEHGE	VCPAGWKPGS	DTIKPNVDDS	KEYFSKHN				

The matched peptides cover **40%** (81/198 AA's) of the protein.

Spot 3:

1	11	21	31	41	51	61	71
MASGNARIGK	PAPDFKATAV	VDGAFKEVKL	SDYKGKYVVL	FFYPLDFTFV	CPTEIIAFSN	RAEDFRKLGC	EVLGVSVDSQ
81	91	101	111	121	131	141	151
FTHLAWINTP	RKEGGLGPLN	IPLLADVTRR	LSEDYGVLKT	DEGIAYRGLF	IIDGKGVLRQ	ITVNDLPVGR	SVDEALRLVQ
161	171	181	191				
AFQYTDEHGE	VCPAGWKPGS	DTIKPNVDDS	KEYFSKHN				

The matched peptides cover **29%** (58/198 AA's) of the protein.

Spot 4:

1	11	21	31	41	51	61	71
MASGNARIGK	PAPDFKATAV	VDGAFKEVKL	SDYKGKYVVL	FFYPLDFTFV	CPTEIIAFSN	RAEDFRKLGC	EVLGVSVDSQ
81	91	101	111	121	131	141	151
FTHLAWINTP	RKEGGLGPLN	IPLLADVTRR	LSEDYGVLKT	DEGIAYRGLF	IIDGKGVLRQ	ITVNDLPVGR	SVDEALRLVQ
161	171	181	191				
AFQYTDEHGE	VCPAGWKPGS	DTIKPNVDDS	KEYFSKHN				

The matched peptides cover **40%** (81/198 AA's) of the protein.

Fig. S1. Sequence coverage of analyzed protein hPrx-2 Spots 1 to 6 and band 1. The lower molecular weight species spot 4 to 6 display sequence coverage comparable to that of the highest molecular weight species Spot 1. Red amino acids are covered through PMF. The active sites are marked by gray background.

Spot 5:

1	11	21	31	41	51	61	71
MASGNARIGK	PAPDFKATAV	VDGAFKEVKL	SDYKGKYVVL	FFYPLDFTFV	CPTEIIAFSN	RAEDFRKLGC	EVLGVSVDSQ
81	91	101	111	121	131	141	151
FTHLAWINTP	RKEGGLGPLN	IPLLADVTRR	LSEDYGVLKT	DEGIAYRGLF	IIDGKGVLRQ	ITVNDLPVGR	SVDEALRLVQ
161	171	181	191				
AFQYTDEHGE	VCPAGWKPGS	DTIKPNVDDS	KEYFSKHN				

The matched peptides cover **43%** (86/198 AA's) of the protein.

Spot 6:

1	11	21	31	41	51	61	71
MASGNARIGK	PAPDFKATAV	VDGAFKEVKL	SDYKGKYVVL	FFYPLDFTFV	CPTEIIAFSN	RAEDFRKLGC	EVLGVSVDSQ
81	91	101	111	121	131	141	151
FTHLAWINTP	RKEGGLGPLN	IPLLADVTRR	LSEDYGVLKT	DEGIAYRGLF	IIDGKGVLRQ	ITVNDLPVGR	SVDEALRLVQ
161	171	181	191				
AFQYTDEHGE	VCPAGWKPGS	DTIKPNVDDS	KEYFSKHN				

The matched peptides cover **35%** (71/198 AA's) of the protein.

Band 1 from pull-down experiment:

1	11	21	31	41	51	61	71
MASGNARIGK	PAPDFKATAV	VDGAFKEVKL	SDYKGKYVVL	FFYPLDFTFV	CPTEIIAFSN	RAEDFRKLGC	EVLGVSVDSQ
81	91	101	111	121	131	141	151
FTHLAWINTP	RKEGGLGPLN	IPLLADVTRR	LSEDYGVLKT	DEGIAYRGLF	IIDGKGVLRQ	ITVNDLPVGR	SVDEALRLVQ
161	171	181	191				
AFQYTDEHGE	VCPAGWKPGS	DTIKPNVDDS	KEYFSKHN				

The matched peptides cover **14%** (29/198 AA's) of the protein.

Fig. S2. (continued)

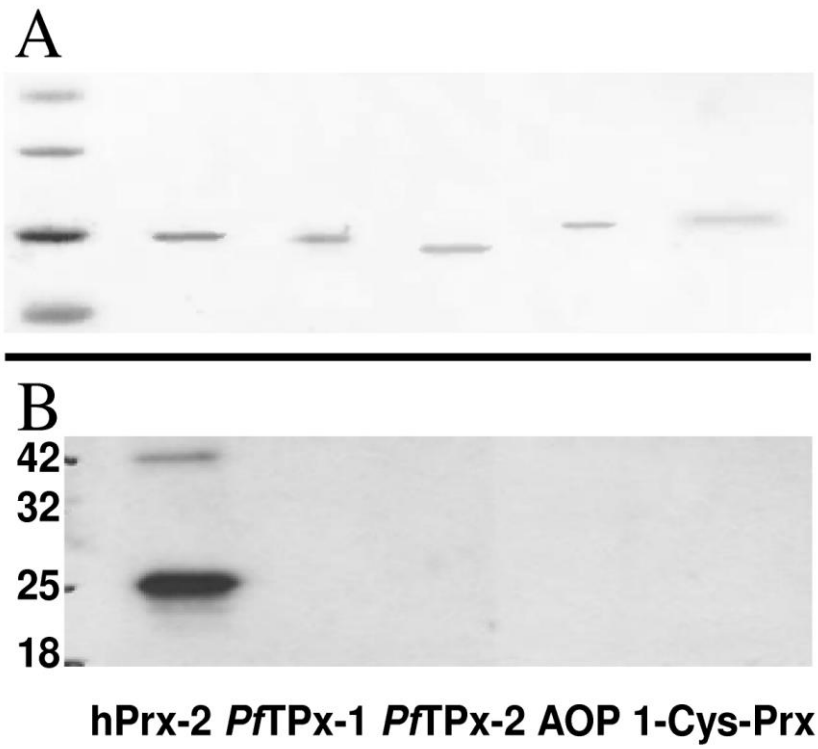


Fig. S2. (*A* and *B*) Protein immunobloting analysis with recombinant peroxidases separated by 1-dimensional SDS/PAGE. The amount of 0.3 μg of each protein was applied and probed with anti-hPrx-2 antibody, recognizing the peptide L^{103}LADVTRRLSED^{114} of the protein (Axxora). The following proteins were applied: hPrx-2; *Pf*TPx1; *Pf*TPx2; *Pf*AOP; *Pf*-1-Cys Prx. The antibody reacts specifically with the human peroxiredoxin-2 and does not show cross-reactivity with the *P. falciparum* enzymes.

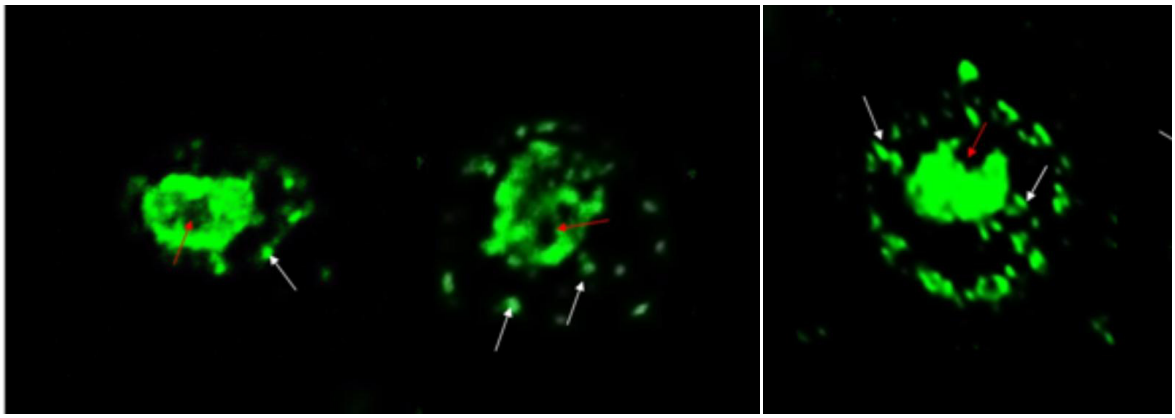


Fig. S3. Confocal laser scanning immunofluorescence microscopy analysis of hPrx-2 in *P. falciparum*-infected erythrocytes. Polyclonal anti-hPrx-2 antibody recognizing the peptide L^{103}LADVTRRLSED^{114} of the protein (Axxora) was used as primary antibody. Anti-hPrx-2-staining is intense inside the parasite, confirming the presence of the human protein inside the *P. falciparum* trophozoites. Furthermore, a clear staining of the Maurer's clefts is visible (*green*). Examples for MCs are marked with white arrows; the FVs are marked with red arrows.

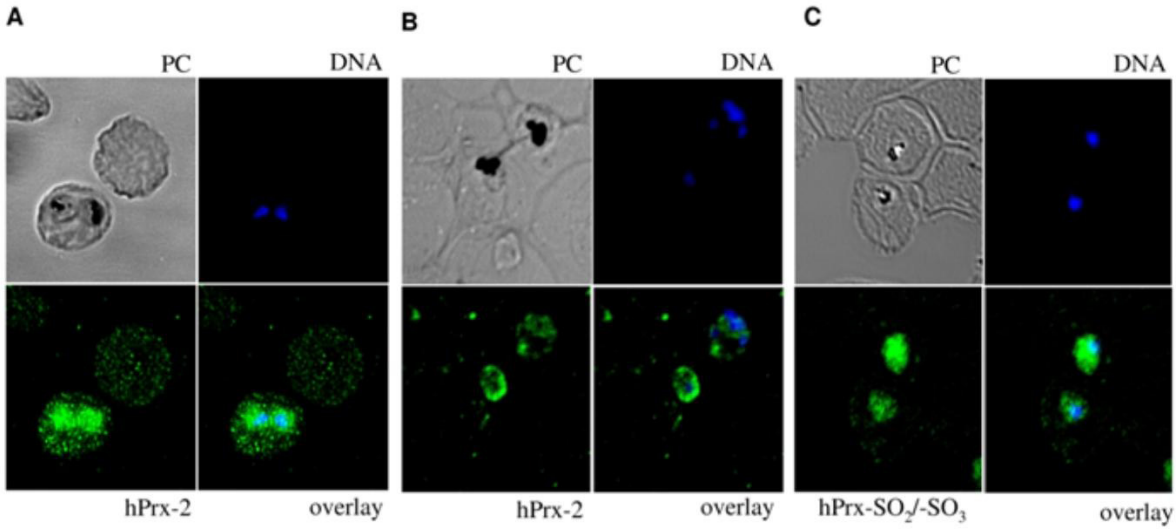


Fig. S4. Confocal laser scanning immunofluorescence microscopy analysis of hPrx-2 in *P. falciparum*-infected erythrocytes with 3 different antibodies against hPrx-2. (*A*) Doubly infected erythrocyte. Polyclonal anti-hPrx-2 antibody recognizing the peptide L^{103}LADVTRRLSED^{114} (Axxora) was used as primary antibody. Anti-hPrx-2-staining is intense inside the parasite confirming the presence of the human protein inside the *P. falciparum* trophozoites (*green*). (*B*) Anti-hPrx-2-stained trophozoite-infected erythrocyte. Polyclonal anti-hPrx-2 antibody directed against the whole protein (kindly provided by L. Poole) was used as primary antibody. The parasite is intensely stained, showing the presence of the hPrx-2 in the parasite's cytoplasm. The FV of the parasite is not stained. (*C*) Two anti-hPrx-2-stained trophozoite-infected erythrocytes. Polyclonal anti-Prx-SO3 recognizing the over-oxidized active site of peroxiredoxins (Acris Antibodies) was used as primary antibody. The parasites are intensely stained, showing the presence of the hPrx-2 in the parasite's cytoplasm. In all images, ''DNA'' indicates staining of host cell and parasite nuclei with Hoechst DNA dye. ''Overlay'' represents an overlay of the 2 images. ''Phase contrast'' (PC) shows the location of the parasitic FVs containing hemozoin (*dark pigment*).

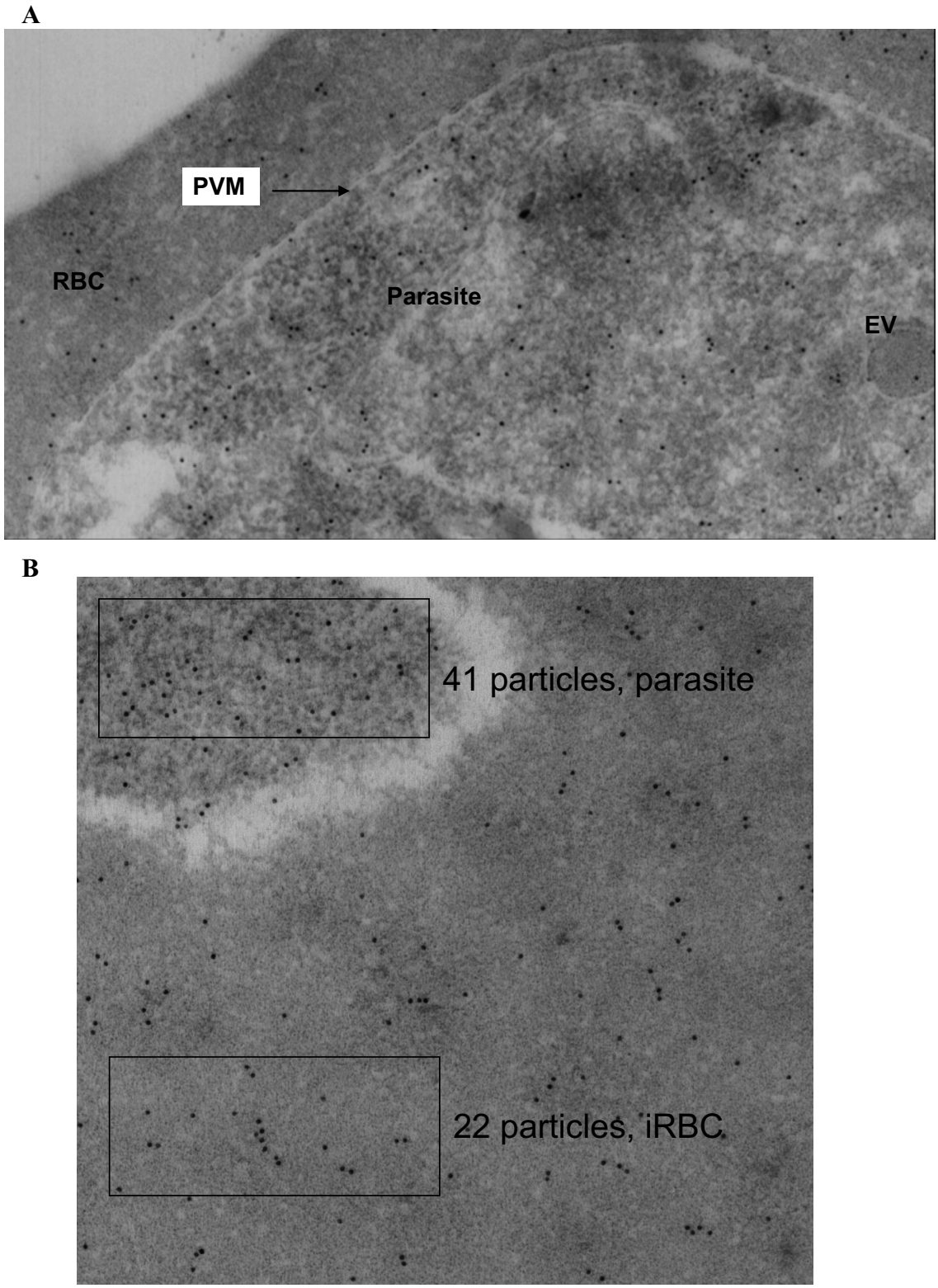


Fig. S5. (*A*) IEM analysis of hPrx-2 of a *P. falciparum*-infected RBC. A polyclonal antibody recognizing the peptide L^{103}LADVTRRLSED^{114} of the human protein was used. Anti-hPrx-2-immunogold staining displays the accumulation of the human protein inside the *P. falciparum* trophozoite cytoplasm (*black dots* = gold particles). Staining is also observed in the RBC cytosol and in an endocytic vesicle (EV). The PVM (*black arrow*) and the parasitophorous vacuole are not stained. (*B*) Example for relative quantitation of immunogold-labeled hPrx-2 in parasite and infected RBC.

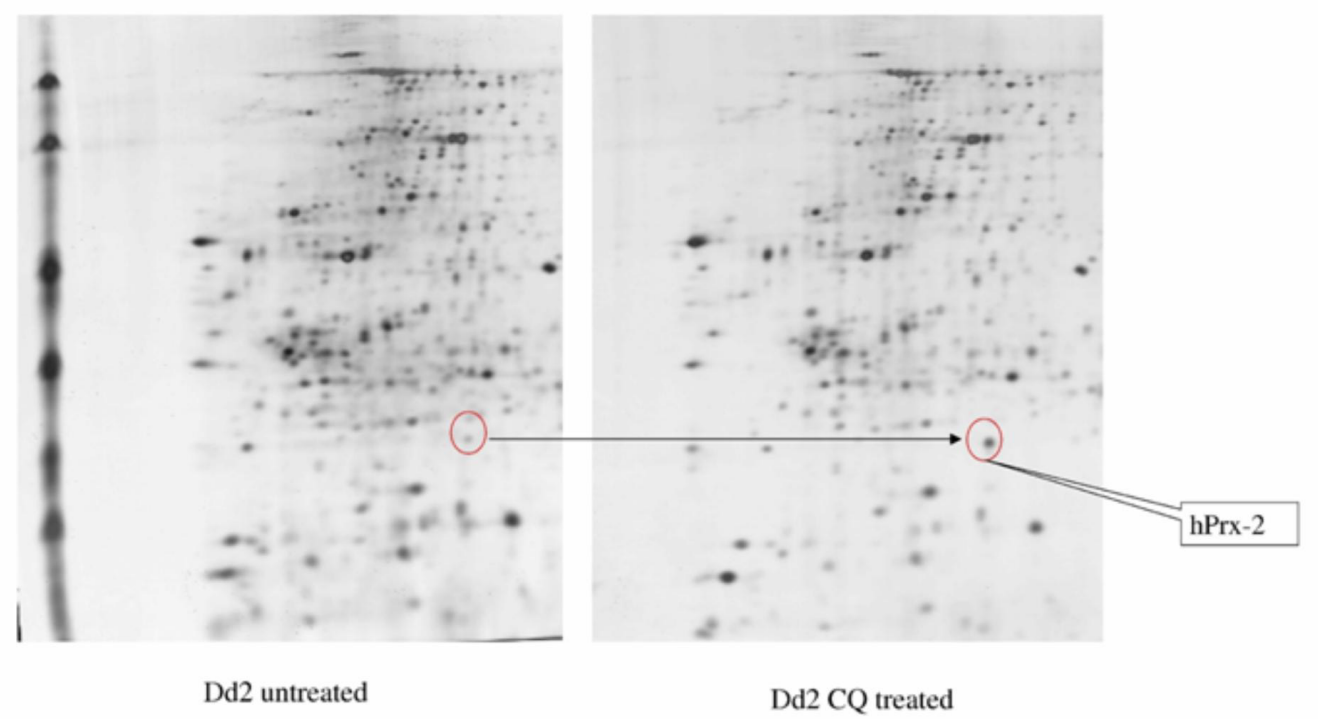
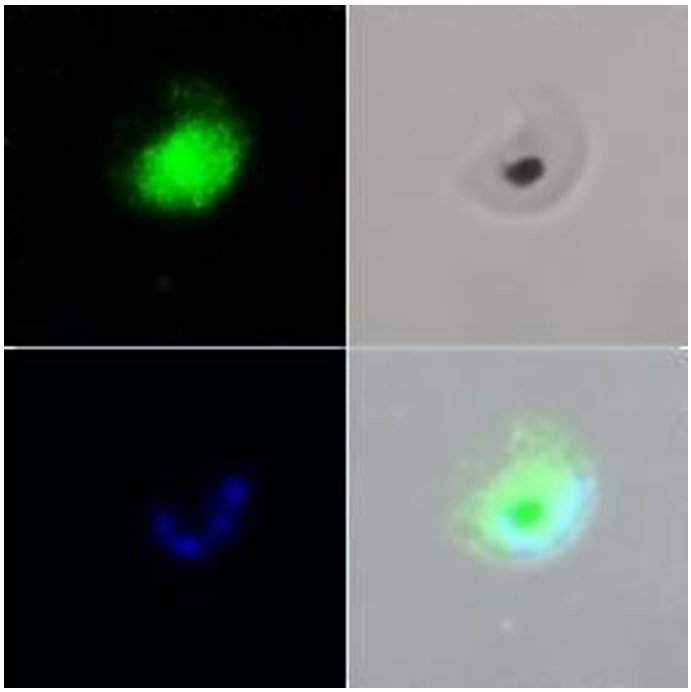


Fig. S6. Regulation of Spot 1 hPrx-2 upon treatment with CQ in *P. falciparum* strain Dd2 as an example where a larger part of the whole gel is shown. The total protein expression pattern as captured by 2DE does not change significantly. The dominant change is the enhanced abundance of hPrx-2.



Movie S1. hPrx-2 import into *Plasmodium* as visualized by indirect IFA with confocal microscopy. The movie displays *z*-axis resolution, showing that hPrx-2 is localized inside the parasite cell and not on the surface or in the parasitophorous vacuole.

Movie S1 (AVI)

Table S1. Summary of peptide mass fingerprint identifications

	Protein	Sequence coverage	Masses matched	$MW_{theor.}$	$pI_{theor.}$	$MW_{app.}$	$\sim pI_{app.}$
Spot 1	hPrx-2	45%	7/100	21.7 kDa	5.7	22 kDa	5.7
Spot 2	hPrx-2	40%	13/100	21.7 kDa	5.7	18 kDa	5.5
Spot 3	hPrx-2	29%	5/100	21.7 kDa	5.7	17 kDa	5.3
Spot 4	hPrx-2	40%	9/100	21.7 kDa	5.7	16 kDa	5.5
Spot 5	hPrx-2	43%	13/100	21.7 kDa	5.7	16 kDa	5.6
Spot 6	hPrx-2	35%	7/100	21.7 kDa	5.7	16 kDa	5.7
Spot 7	*Pf*-1-Cys-Prx	40%	9/70	25.1 kDa	6.3	25 kDa	6.8
Band 1	hPrx-2	14%	3/41	21.7 kDa	5.7	22 kDa	-

Spots 1 to 6 (Fig. 1*A*) were cut from Coomassie-stained 2DE-gels and tryptically digested. MALDI-MS revealed the presence of the human peroxiredoxin-2 (hTPx1, hPrx-2) in Spots 1 to 6 and the *Pf*-1-Cys-Prx in Spot 7. Band 1 was cut from a 1-dimensional SDS-Gel on which the DTT-eluted fraction of the $PfTrx^{C33S}$ pull-down was separated.

Table S2. Enrichment of hPrx-2 in comparison to Hb in parasite extract, compared to extract of lysed-infected RBCs as determined by LC-MS analyses

	Spec counts: parasite	Ratio hPrx-2/Hb	Spec counts: Lysate of iRBC	Ratio hPrx-2/Hb	Enrichment of hPrx-2 compared to Hb
Hb β	403		6,037		
hPrx-2	28	0.069	9	0.001	47
Hb α	617		1,734		
hPrx-2	28	0.045	9	0.005	9
Hb δ	6		149		
hPrx-2	28	4.667	9	0.060	77
					Ø44

Table S3. Comparison of FV preparations by determining acetylcholine esterase (AcChE) activity

	Saliba et al., 1998[a]	Present study
AChE activity of vacuolar preparations [μmol/h $\times$ mg protein]	0.66	0.93
AChE activity of erythrocte membranes [μmol/h $\times$ mg protein]	11.6	15.3
Ratio AChE activity of erythrocytic membranes/vacuoles	17.6	16.4
Erythrocyte membrane protein/vacuole protein [ng/μg]	60	61

[a]Saliba KJ, Folb PI, Smith PJ (1998) Role for the *Plasmodium falciparum* digestive vacuole in chloroquine resistance. *Biochem Pharmacol* 56:313–320. Parasite FVs were prepared as described in Jackson et al. [Jackson KE, et al. (2004) Food vacuole-associated lipid bodies and heterogeneous lipid environments in the malaria parasite, *Plasmodium falciparum*. *Mol Microbiol* 54:109–122.] The degree of contamination of FV preparations with host material was monitored by measuring acetylcholine esterase activity. The table shows a very good reproducibility of the preparation resulting in a deduced amount of only 61 ng of erythrocyte membrane protein per microgram of vacuolar protein.